Tent5a Cas9-KO Strategy

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Reviewer: Xiaojing Li

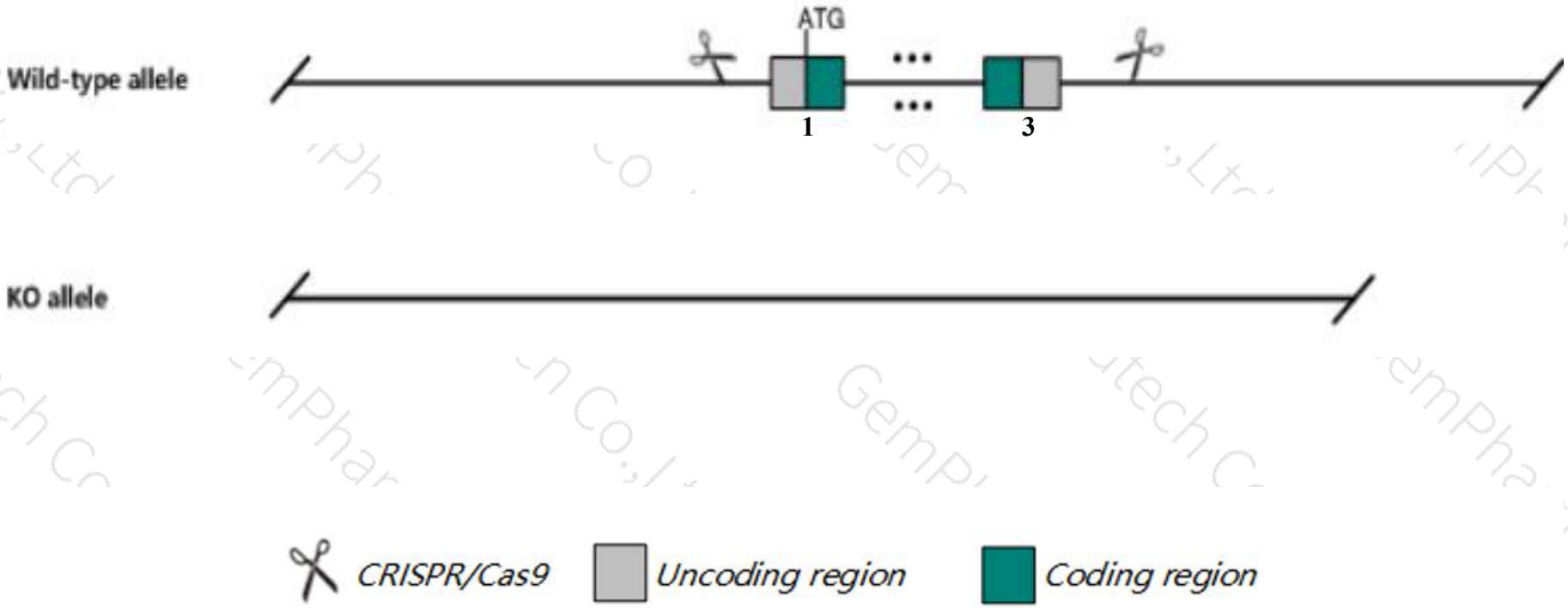
Design Date: 2020-8-28

Project Overview

Project Name	***Tent5a***
Project type	**Cas9-KO**
Strain background	**C57BL/6JGpt**

Knockout strategy

This model will use CRISPR/Cas9 technology to edit the *Tent5a* gene. The schematic diagram is as follows:



Technical routes

➢ The *Tent5a* gene has 2 transcripts. According to the structure of *Tent5a* gene, exon1-exon3 of *Tent5a-201*(ENSMUST00000034802.14) transcript is recommended as the knockout region.The region contains all of the coding sequence.Knock out the region will result in disruption of protein function.

➢ In this project we use CRISPR/Cas9 technology to modify *Tent5a* gene. The brief process is as follows: CRISPR/Cas9 system were microinjected into the fertilized eggs of C57BL/6JGpt mice.Fertilized eggs were transplanted to obtain positive F0 mice which were confirmed by PCR and sequencing. A stable F1 generation mouse model was obtained by mating positive F0 generation mice with C57BL/6JGpt mice.

Notice

➢ According to the existing MGI data,homozygotes for an ENU-induced allele show partial lethality, high alkaline phosphatase (ALP) activity, short stature, and limb, long bone, rib, pelvis and skull anomalies, with absent trabeculae and reduced cortical thickness in long bones. Heterozygotes show high ALP activity but no other defects.

➢ The *Tent5a* gene is located on the Chr9. If the knockout mice are crossed with other mice strains to obtain double gene positive homozygous mouse offspring, please avoid the two genes on the same chromosome.

➢ This strategy is designed based on genetic information in existing databases.Due to the complexity of biological processes,all risk of the gene knockout on gene transcription, RNA splicing and protein translation cannot be predicted at the existing technology level.

Gene information（NCBI）

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Tent5a terminal nucleotidyltransferase 5A [Mus musculus (house mouse)]

Gene ID: 212943, updated on 13-Mar-2020

Summary

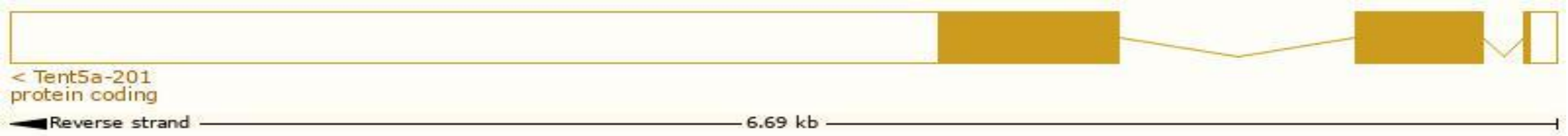
Official Symbol	Tent5a provided by MGI
Official Full Name	terminal nucleotidyltransferase 5A provided byMGI
Primary source	MGI:MGI:2670964
See related	Ensembl:ENSMUSG00000032265
Gene type	protein coding
RefSeq status	VALIDATED
Organism	Mus musculus
Lineage	Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi; Mammalia; Eutheria; Euarchontoglires; Glires; Rodentia; Myomorpha; Muroidea; Muridae; Murinae; Mus; Mus
Also known as	BAP014, D930050G01Rik, Fam46a
Expression	Broad expression in colon adult (RPKM 11.3), placenta adult (RPKM 10.4) and 25 other tissuesSee more
Orthologs	human all

Transcript information（Ensembl）

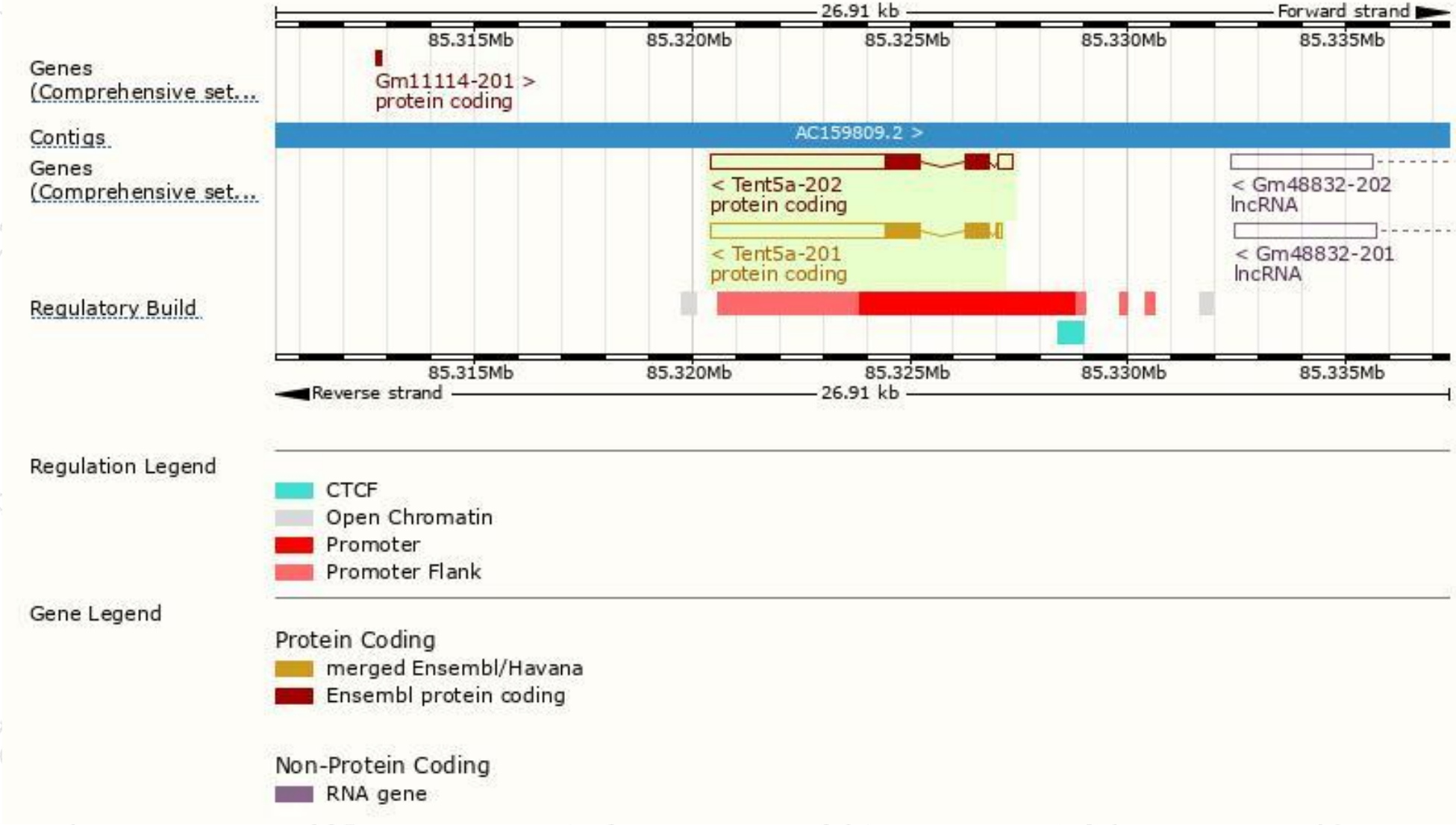
The gene has 2 transcripts,all transcripts are shown below:

Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
Tent5a-202	ENSMUST00000187711.1	5648	428aa	Protein coding	CCDS81046	A0A087WS27	TSL:1 GENCODE basic APPRIS ALT2
Tent5a-201	ENSMUST00000034802.14	5479	447aa	Protein coding	CCDS52875	D3Z5S8	TSL:3 GENCODE basic APPRIS P3

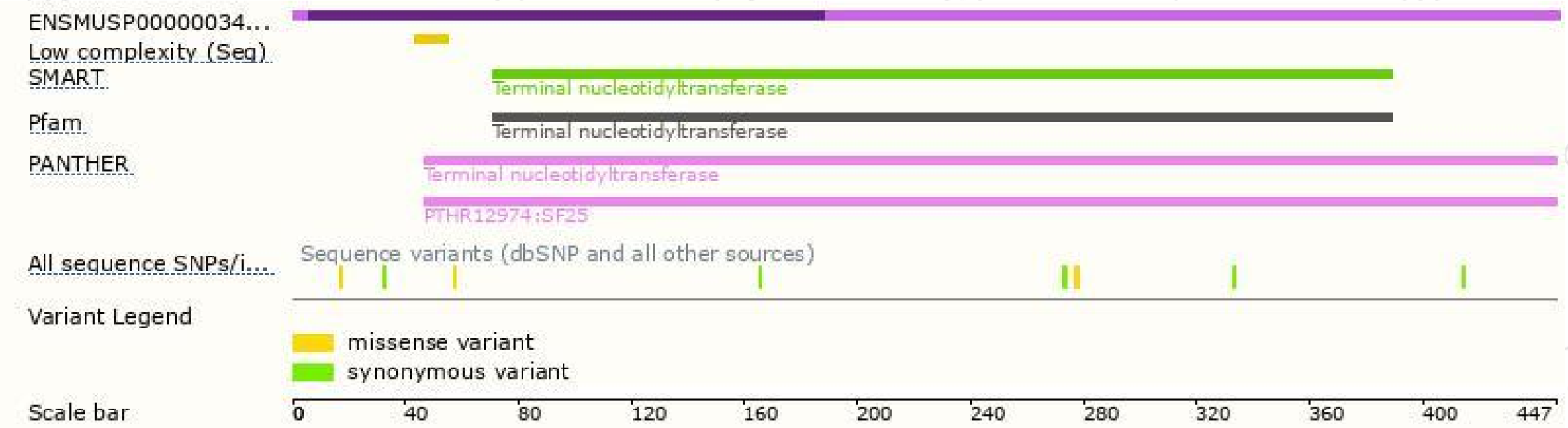
The strategy is based on the design of *Tent5a-201* transcript,the transcription is shown below:



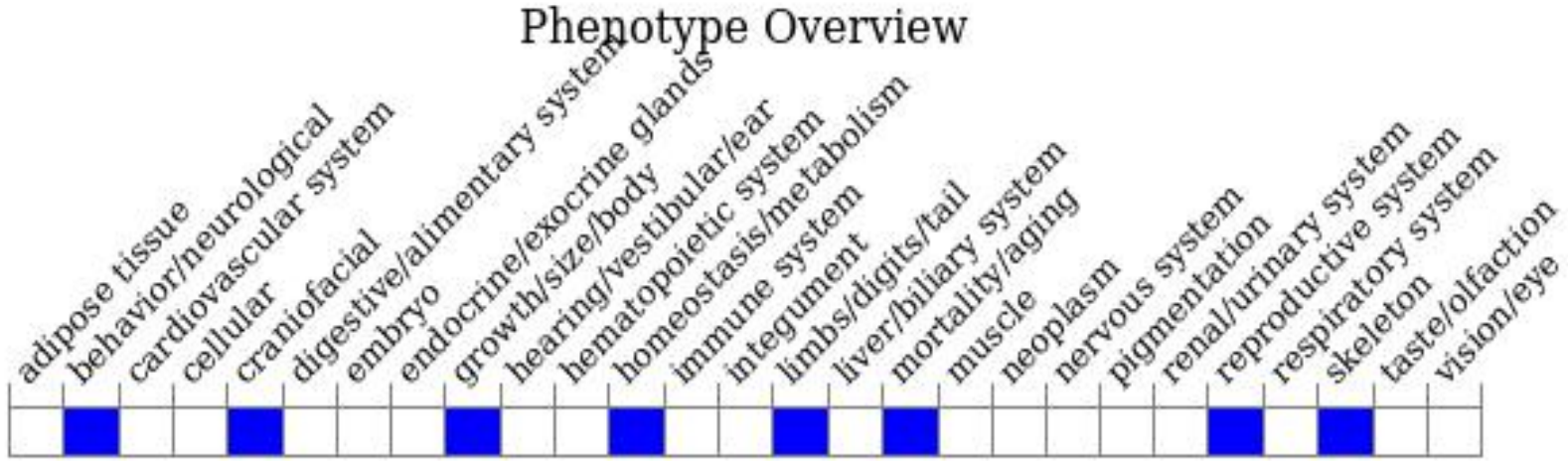
Genomic location distribution



Protein domain



Mouse phenotype description(MGI)



Phenotypes affected by the gene are marked in blue.Data quoted from MGI database(http://www.informatics.jax.org/).

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If you have any questions, you are welcome to inquire.

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